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Fitness consequences of pheromone production and host selection strategies in a tree-killing bark beetle (Coleoptera: Curculionidae: Scolytinae)

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Abstract Timing of arrival at a resource often determines an individual's reproductive success. Tree-killing bark beetles can reproduce in healthy trees by attacking in adequate numbers to overcome host defences that could otherwise be lethal. This process is mediated by aggregation and antiaggregation pheromones. Beetles that arrive early in such a "mass attack" must contend with undiminished tree defences, and produce enough pheromones to attract more beetles, but have a head start on gallery construction and egg-laying. Beetles that arrive late may be impeded by competition and diminishing availability of phloem, but should experience fewer costs associated with pheromone production and battling tree defences. We investigated relationships between timing of arrival, body size, pheromone production and fitness in the southern pine beetle, *Dendroctonus frontalis*. In field experiments, we captured beetles that arrived early (pioneers) and late on slash pine trees, *Pinus elliottii*, and measured pheromone amounts in their hindguts. We marked gallery entrances of beetles as they landed on a tree and measured their reproductive success after the attack terminated. We found no difference in body size or pheromone amounts between early and late arrivers. Most beetles arrived at the middle of the attack

sequence, and excavated longer galleries per day than early arrivers. The number of offspring produced per day by beetles that established galleries midway through mass attack was higher than those that arrived early or very late in the sequence. Our results suggest that beetles do not exhibit adaptive phenotypic plasticity in pre-landing pheromone production, depending on the extent of previous colonisation of a host. Rather, it appears that stabilising selection favours beetles that attack in the middle of the sequence, and contributes to attack synchrony. Synchronous attack on trees is essential before population booms characteristic of tree-killing bark beetles can occur in nature.

Keywords Southern pine beetle · Cooperation · Timing of arrival · Stabilising selection · Attack synchrony

Introduction

The evolution of cooperation among unrelated individuals despite competition and opportunities to cheat is a perplexing question in evolutionary biology (Trivers 1971; Axelrod and Hamilton 1981; Dugatkin 1997; Avilés et al. 2002; Foster 2004). Cooperative behaviour is essential when survival and reproduction depend on obtaining a resource that may be inaccessible to solitary individuals (Caraco and Wolf 1975; Berryman et al. 1985; Marzluff and Heinrich 1991; Avilés and Tufino 1998). Timing of arrival of individuals at a resource that is shared by the group for feeding and breeding also determines relative reproductive success among members (Blums et al. 2005; Dickerson et al. 2005). Fitness in tree-killing bark beetles (Coleoptera: Curculionidae: Scolytinae), is contingent on their ability to aggregate, as combined effort of group members is required to overcome defensive responses of the tree and kill it so that

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beetles may feed and reproduce in galleries they excavate in the phloem (Raffa and Berryman 1983). Beetles arrive at a suitable tree, and produce potent aggregation pheromones that attract more conspecifics to the tree (Wood 1982; Raffa et al. 1993). Sufficient quantities of aggregation pheromones must be produced by the group to attract enough beetles to kill the tree (Raffa and Berryman 1987; Raffa 2001; Aukema and Raffa 2004). Once the tree is under “mass attack”, antiaggregation pheromones are produced by beetles that terminate attack, prevent overcrowding, and reduce competition for phloem resource on which larvae feed (Wood 1982; Raffa et al. 1993).

Beetles incur differential risks and benefits depending on their timing of arrival on the tree. “Pioneer” beetles that are the first to arrive and initiate mass attack have to contend with resistance mechanisms of the tree, and must produce sufficient quantities of aggregation pheromones to attract a lethal number of additional beetles to the tree (Raffa and Berryman 1983). However, by getting a head start on gallery construction and egg-laying, they may produce more offspring per capita, that have shorter development time and therefore a competitive advantage over those of late arrivers. Because tree defences would already be exhausted, beetles that arrive late in mass attack are more likely to survive initial entry into the phloem than pioneers. We predict that late arrivers would not benefit by attracting further conspecifics to the tree, and, consequently, would not produce as much pheromone as pioneers. Late arrivers, however, would produce fewer and less fit offspring than pioneers, because much of the available phloem would already be occupied by the brood of beetles that arrive early in the attack sequence.

The southern pine beetle, *Dendroctonus frontalis* Zimmermann, is a major killer of pines in the southern United States (Drooz 1985; Price et al. 1998). Evidence suggests that five main pheromones function in aggregation and antiaggregation. Females produce the aggregation pheromone frontalin when they land on a tree, and initiate galleries, attracting both males and females (Payne et al. 1978). *trans*-Verbenol is produced predominantly by females and synergises the attraction of frontalin (Payne et al. 1978). Males pair with females and produce *endo*-brevicomin (Pitman et al. 1969), which further facilitates aggregation at low concentrations, but acts as an antiaggregation pheromone at high concentrations (Vité et al. 1985; B.T. Sullivan, 2004, personal communication). Both sexes produce antiaggregation pheromones verbenone (predominantly males) and myrtenol that function in terminating aggregation (Payne et al. 1978), and reducing intraspecific competition. In studies of the chemical ecology and management of *D. frontalis*, the roles of frontalin and verbenone have been elucidated, particularly from a management perspective (Clarke et al. 1999), while those of *endo*-brevicomin, *trans*-verbenol and myrtenol are less clear. Our study explores whether different “strategies” exist

regarding pheromone production in *D. frontalis*, and does not evaluate their mechanistic functions.

Body size also influences fitness in bark beetles. Large beetles can fly further, sire more offspring (Reid and Roitberg 1995; Robertson and Roitberg 1998), lay more eggs (Reid 1962; McGhehey 1971; Clarke et al. 1979), survive harsh environmental conditions better (Safaryik 1976) and produce more pheromones (Pureswaran and Borden 2003) than small ones.

Bark beetles in a population vary considerably in the amount of pheromone they produce (Hunt et al. 1986; Birgersson et al. 1988; Miller et al. 1989; Zhang et al. 2000; Pureswaran and Borden 2003), and variation among individuals is greater than that seen in solitary callers like moths (Schlyter and Birgersson 1989). Two pheromones of *D. frontalis*, frontalin and *endo*-brevicomin are synthesised *de novo* (Vanderwel et al. 1992; Barkawi et al. 2003) and must involve some energetic cost. The other pheromones, *trans*-verbenol, verbenone and myrtenol are oxidation products of host monoterpenes (Hughes 1973, 1975; Renwick et al. 1976), and may be less metabolically costly than pheromones synthesised *de novo*. Other costs of pheromone production include attracting predators (Reeve 1997; Aukema and Raffa 2004) and competing heterospecific bark beetles (Svihra et al. 1980) that respond positively to pheromones (Ayres et al. 2001; Dahlgren et al. 2004). Although *D. frontalis* is one of the most intensively studied tree-killing bark beetles in North America, investigations on the behaviour of individual beetles have been surprisingly limited.

It has been hypothesised that mass aggregation in tree-killing bark beetles might foster the evolution of “cheating” (Birgersson et al. 1988; Raffa 2001; Aukema and Raffa 2004). We hypothesise that beetles that arrive at the end of mass attack can potentially escape the costs associated with pheromone production, and potentially lethal amounts of host resin, and still achieve reproductive success, especially in epidemic conditions in which beetle populations are large enough to overcome defences of vigorous hosts. The size of individuals and pheromone production rates could influence the sequence in which they attack a tree, particularly if pheromone production is an adaptively plastic trait (Johansson et al. 2005). Alternatively, beetles may be able to regulate the amount they produce depending on the condition of the tree they choose to attack. Under either of the above scenarios, we would expect varying fitness consequences to be associated with the sequence in which beetles attack a tree.

We tested the following theoretical predictions using *D. frontalis* as our model system: (1) pioneer beetles will be larger and produce more pheromone than late arrivers on landing on the tree, and (2) the costs and benefits associated with being pioneers or late arrivers will tend to equalise fitness differences between them.

Materials and methods

Timing of attack, beetle size and pheromone production

Collection and treatment of beetles

Studies were conducted within a stand of slash pine, *Pinus elliotii* Engelm., infested with *D. frontalis* within the Chickasawhay Ranger District of the DeSoto National Forest in southeastern Mississippi. Trees were 22 years of age, 19 ± 4 (\pm SD) cm DBH, and 19.5 ± 2.6 m height, with a basal area of pine (BAF $10 \times$ prism) of 30 ± 7 m²/ha. As part of another study, we measured naturally occurring *D. frontalis* landings every 3 days on each tree within and around the infestation, and mapped the progression of attacks through the stand. This permitted us to identify trees that were at different stages of attack. From 31 July to 2 August 2004, beetles from the same dispersing cohort were intercepted in flight with funnel traps (Lindgren 1983) just before they would have landed on one of two pre-selected trees: (1) a tree on which attacks had started within the previous 2–3 days and was still exuding some oleoresin from sites of attack (suitable for putative pioneer beetles), or (2) a nearby (≈ 4 m), size-matched, tree that had been sustaining attacks for about 10 days and whose resin defences were exhausted (suitable for putative late arrivers). These two sets of landing beetles, pioneers and late arrivers, were compared with respect to their size and pheromone production (see next section). We also collected a sample of beetles that were allowed to land and begin boring oviposition gallery (boring beetles). On 7 and 8 August we marked newly formed gallery entrances on a tree that had come under attack during the previous 2–3 days. On 10–11 August, we removed the male–female pairs within each of these galleries and measured their size, the length of their galleries and pheromone production.

Analyses of size and pheromone amounts in individual beetles

Beetles were sexed (Osgood and Clark 1963), placed in individual Petri dishes with moist paper towels and transported to the laboratory. Body length and width of the head capsule were measured as indicators of body size using a dissection microscope with an ocular micrometer. Hindguts and posterior portions of the midguts of beetles were dissected on the day of capture using sharp forceps and placed in 50 μ l of hexane containing 3.5 ng/ μ l of heptyl acetate as an internal standard (Sullivan 2005). Samples were frozen until analysed on an Agilent 6890–5973 coupled gas chromatograph–mass spectral detector (GC–MS) operating with an HP-INNOWax (Agilent Technologies; 60 m \times 0.25 mm \times 0.25 μ m film) column. The temperature program was 40 °C for 1 min, 16° C/min to 80 °C, then 7 °C per min to 230 °C and held for 10 min. The flow of helium was

1.0 ml/min, the injector and detector ports were held at 200 and 240 °C respectively. Pheromone amounts were quantified using response curves calculated from analyses of a dilution sequence of known quantities of synthetic standards using MSD ChemStation Standalone software (G1701DA Version D.00.00.38, 1989–2001, Agilent Technologies, Palo Alto, CA), and their identifications were confirmed by comparing retention times with known standards. Five major pheromones of *D. frontalis*, frontalin, *endo*-brevicomin, *trans*-verbenol, verbenone and myrtenol (Payne et al. 1978) were quantified.

Timing of attack and beetle fitness

A single *P. elliotii* (25.5 cm diameter at 1.5 m height) was monitored over a 3-week period and landing and gallery initiation by beetles was recorded. The first evidence of beetles landing on the tree was noted on 26 July 2004, but the first sign of gallery initiation was not evident until 2 August. Gallery entrances of 274 pairs of attacking beetles on a 1.25-m-long section, 1.2 m from the ground, were marked daily as they appeared between 1 August and 16 August. Beetles were classified into four groups, early (1–4 August), mid (5–8 August), late (9–12 August), and very late (13–16 August) arrivers, depending on their timing of gallery initiation on the tree. On 18 August, after the attack had ceased, the bark was stripped, and the fitness of the attacking beetles was assessed by measuring gallery length, and counting the number of egg niches and larvae from 100 galleries.

Statistical analyses

Pheromone amounts were transformed by $\log_{10}(x+1)$ to improve normality and homoscedasticity. Differences in size between landing pioneers and late arrivers were analysed by multivariate analysis of variance. Differences in pheromone amounts related to (1) host selection strategy (pioneers vs. late arrivers), (2) stage of attack (landing vs. boring beetles), (3) sex (females vs. males), (4) interactions between strategy and sex and (5) stage and sex were analysed using a priori linear contrasts following multivariate analysis of variance. Principal components analysis of the correlation matrix of log-transformed amounts of all five pheromones was employed to visually compare beetle groups with respect to their pheromone production.

One-way analyses of variance (ANOVA) and the Ryan-Einot-Gabriel-Welsh (REGW) multiple comparisons tests (Day and Quinn 1989) were performed to determine if there were differences in the amounts of the five pheromones produced by beetles at every stage of attack, (1) landing females, (2) landing males, (3) boring females, and (4) boring males. The REGW test uses a multiple stage approach and is a powerful test for all pairs comparisons because it controls the maximum

experiment-wise error rate under any complete or partial hypothesis (Day and Quinn 1989; Reeve and Strom 2004). Data from pioneers and late arrivers were pooled for this analysis, and comparisons were among pheromones within each sex and stage of attack. Correlation analyses were performed to determine whether the amounts of different pheromones produced by the same beetles were correlated with each other, and whether the amounts produced by females were correlated with the males they were paired with in galleries.

Effects of timing of attack and gallery length on fitness were analysed using one-way ANOVA and the REGW multiple comparisons test. A linear regression analysis was performed with gallery length excavated per day as the independent variable and number of offspring produced per day as the dependent variable. All analyses were performed using SAS Institute Inc. version 8.2 (1999–2001) statistical software, and $\alpha=0.05$. The sequential Bonferroni adjustment (Rice 1989) was used to lower the α -value to account for the number of correlation analyses performed.

Results

Timing of attack, beetle size and pheromone production

There was no difference in body size of pioneers vs. late arrivers: for females, body length \pm SE = 3.70 ± 0.09 vs. 3.62 ± 0.07 mm and head capsule width = 1.05 ± 0.03 vs. 1.06 ± 0.03 mm; or for males, body length = 3.62 ± 0.06 vs. 3.53 ± 0.10 mm and head capsule width = 1.04 ± 0.03 vs. 1.03 ± 0.03 mm ($n=20$ – 22 beetles per group; MANOVA: $F_{(2,79)}=1.26$, $P=0.29$).

There were also no differences in pheromone amounts between landing pioneers and late arrivers, although there were conspicuous differences between sexes (Tables 1, 2; Fig. 1a). Compared to landing males, landing females contained at least tenfold greater amounts of frontalin and *trans*-verbenol, but far less myrtenol and largely undetectable amounts of verbenone and *endo*-brevicomin. Pheromone production of both sexes tended to change within a few days of boring within the phloem (landing vs. boring in Tables 1, 2; Fig. 1b). Boring females had less frontalin and *trans*-

verbenol, but more verbenone and myrtenol in their hindguts than landing females. Boring males had less *endo*-brevicomin, verbenone and myrtenol, but more frontalin than landing males. These changes in pheromone production after landing largely eliminated the initial differences between sexes (Table 1, Fig. 1b). Pheromones that are synthesised *de novo* (frontalin and *endo*-brevicomin) were in much lower amounts ($\approx 1\%$ on average) than those derived from monoterpene precursors found in the host (*trans*-verbenol, verbenone and myrtenol), although the coefficient of variation (SD/mean) among individuals was similarly high for both classes of pheromones ($> 140\%$).

Individual beetles that contained larger amounts of one pheromone tended to also contain larger amounts of other pheromones (Table 3). For example, there were significant positive correlations among *trans*-verbenol, frontalin, and myrtenol for landing females (Table 3, upper matrix), among verbenone, *endo*-brevicomin, and myrtenol for landing males (Table 3, second matrix), and among *trans*-verbenol, verbenone, and myrtenol for boring beetles of both sexes (Table 3, lower matrices). In boring beetles that were paired in galleries, females with high amounts of verbenone tended to be paired with males with high amounts of verbenone and myrtenol in their hindguts (Table 4). However, paired females and males were not assorted either by body length ($r=0.45$, $P=0.10$) or head capsule width ($r=0.07$, $P=0.81$). There was no correlation between beetle size and the amount of pheromone in the hindguts of either males or females in any category ($P>0.05$ in all cases, data not shown). There was also no correlation between gallery length and amount of pheromone in either boring males or females ($P>0.05$, for all pheromones).

Timing of attack and beetle fitness

Of the 247 pairs of beetles whose galleries were marked, a majority (50.4%) initiated attack between 5 and 8 August in the middle of the attack sequence (Fig. 2a), while 17.2% initiated attack early in the sequence, 24.5% attacked late, and 8% attacked very late in the sequence. Beetles that initiated attack in the middle of the sequence had longer total galleries when the attack

Table 1 Means and standard errors (in parentheses) of pheromone amounts (ng/hindgut) in *D. frontalis*, $n=20$ – 22

| Pheromones | Pioneers | | | | Late arrivers | |
|-------------------------|----------------|-----------------|---------------|---------------|----------------|----------------|
| | Landing | | Boring | | Landing | |
| | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ |
| Frontalin | 1.63 (0.55) | 0.05 (0.05) | 0.36 (0.11) | 0.23 (0.11) | 4.99 (3.33) | 0.00 (0.00) |
| <i>endo</i> -Brevicomin | 0.00 (0.00) | 1.48 (0.58) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 3.33 (1.81) |
| <i>trans</i> -Verbenol | 163.71 (54.08) | 17.08 (6.53) | 39.53 (24.60) | 21.19 (14.65) | 147.66 (44.58) | 14.99 (10.93) |
| Verbenone | 0.00 (0.00) | 539.03 (246.36) | 7.57 (3.35) | 27.60 (21.30) | 0.10 (0.10) | 259.93 (92.97) |
| Myrtenol | 5.18 (1.43) | 36.49 (12.79) | 11.98 (4.73) | 17.46 (8.22) | 3.96 (1.08) | 45.32 (13.55) |

Table 2 Effects of arrival strategy, stage of attack and sex of *D. frontalis* on pheromone production

| | | <i>F</i> -statistics | | | | | |
|----------|----------------------------|----------------------|-------------------------|------------------------|-----------|----------|----------|
| | | Frontalin | <i>endo</i> -Brevicomin | <i>trans</i> -Verbenol | Verbenone | Myrtenol | MANOVA |
| Strategy | Pioneers vs. Late arrivers | 0.01 | 0.94 | 0.16 | 0.02 | 0.01 | 0.26 |
| Stage | Landing vs. Boring | 1.02 | 5.88* | 12.69*** | 6.11* | 0.70 | 4.8*** |
| Sex | Female vs. Male | 20.49*** | 22.13*** | 55.32*** | 72.36*** | 17.40*** | 53.35*** |
| | Strategy \times sex | 0.20 | 0.94 | 1.63 | 0.00 | 0.61 | 0.83 |
| | Stage \times sex | 5.18* | 5.88* | 11.97*** | 20.84*** | 6.48* | 13.68*** |

Results of a priori linear contrasts following MANOVA ($df=5, 114$)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

on the tree terminated ($F=14.02$, $df=3,96$, $P<0.0001$) than late and very late arrivers, although they were not significantly different from those of early arrivers (Fig. 2b). However, beetles that arrived in the middle of the sequence excavated significantly more galleries per day than those that came in early, but did not differ from late or very late arrivers ($F=7.08$, $df=3,96$, $P=0.0002$; Fig. 2c). As usual for *D. frontalis* (Foltz et al. 1976), the length of oviposition gallery was strongly and linearly related to the number of offspring: offspring = $0.61 \text{ cm} + 0.08$, $r^2=0.68$ (or about 1 egg per 1.6 cm of oviposition gallery). Therefore, beetles that came in at the middle of the attack sequence also produced the most total offspring per capita ($F=17.93$, $df=3,96$, $P<0.0001$; Fig. 3a) on the day the attack terminated,

compared to beetles in any of the other categories. The total number of offspring produced by beetles that arrived early in the sequence did not differ significantly from late arrivers. The number of offspring produced per day by beetles that came in at the middle was about twice as high as early and very late arrivers, although it was not significantly different from late arrivers ($F=11.53$, $df=3,96$, $P<0.0001$; Fig. 3b).

Discussion

Timing of attack, beetle size and pheromone production

Timing of arrival on the tree and beetle size were unrelated to the amount of pheromone produced by landing *D. frontalis*, and did not explain any of the high variation among individuals in the attacking group (Table 2). If selection pressures such as predation, or competition from heterospecific beetles are relatively low at a given point in time, a whole range of phenotypes for quantitative variation in pheromone production can persist in the population.

Our results suggest the absence of adaptive plasticity in pre-landing pheromone production depending on the density of previous attacks on a host, but do not preclude the possibility of phenotypic plasticity with respect to an individual's physiological state such as age, diet, or re-emergence status. In the southern United States, individual *D. frontalis* often re-emerge and attack more than one tree per season (Cooper and Stephen 1978). Re-emerging beetles have been reported to be less attractive than virgin females (Coster 1970), and may have exhausted their physiological potential to produce high amounts of pheromone, accounting for the low pheromone producers in the population. It appears therefore, that alternative phenotypes for pre-landing pheromone production exist in *D. frontalis* before beetles land and bore into trees they choose to attack. Under endemic conditions, however, phenotypes that produce little or no pheromone may be eliminated from the population if they do not produce sufficient pheromone to attract enough beetles to kill the tree.

Our results also reveal that pheromones that are directly derived from host precursors (*trans*-verbenol, verbenone, and myrtenol; Hughes 1973, 1975; Renwick

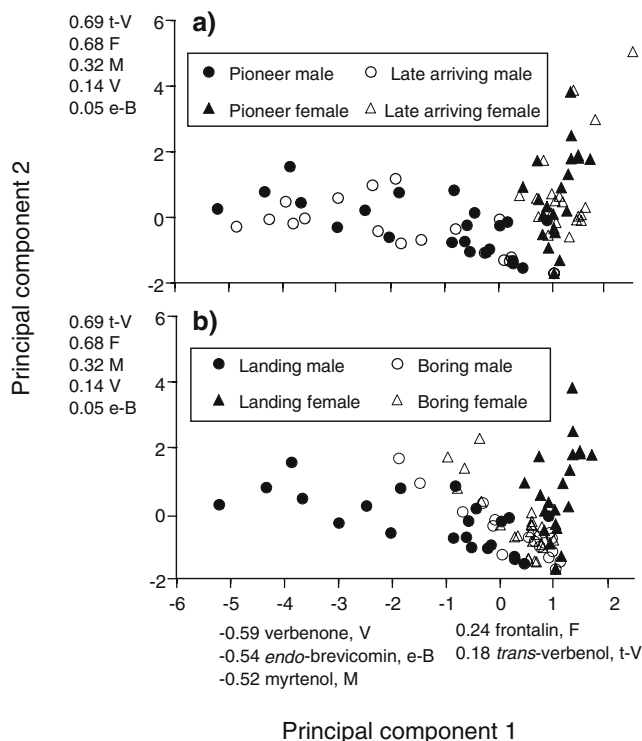


Fig. 1 Principal components analysis of *D. frontalis*, **a** male and female pioneers vs. late arrivers and **b** male and female landing beetles vs. boring beetles. Each point represents one beetle. Axes are labelled with the factor loadings (eigenvectors) for each pheromone. See Table 2 for corresponding MANOVA results

Table 3 Correlation matrices of pheromone production in *D. frontalis* for landing females, landing males, boring females, and boring males

| Pheromones | Frontalin | <i>endo</i> -Brevicomin | <i>trans</i> -Verbenol | Verbenone | Myrtenol |
|----------------------------------|-----------|-------------------------|------------------------|-----------|----------|
| Landing females (<i>n</i> = 41) | | | | | |
| Frontalin | NA | | | | |
| <i>endo</i> -Brevicomin | NA | NA | | | |
| <i>trans</i> -Verbenol | 0.60*** | NA | NA | | |
| Verbenone | NA | NA | NA | NA | |
| Myrtenol | 0.43** | NA | 0.57*** | NA | NA |
| Landing males (<i>n</i> = 41) | | | | | |
| Frontalin | NA | | | | |
| <i>endo</i> -Brevicomin | NA | NA | | | |
| <i>trans</i> -Verbenol | NA | -0.04 | NA | | |
| Verbenone | NA | 0.68*** | 0.38* | NA | |
| Myrtenol | NA | 0.64*** | 0.03 | 0.78*** | NA |
| Boring females (<i>n</i> = 14) | | | | | |
| Frontalin | NA | | | | |
| <i>endo</i> -Brevicomin | NA | NA | | | |
| <i>trans</i> -Verbenol | 0.54 | NA | NA | | |
| Verbenone | 0.64* | NA | 0.85*** | NA | |
| Myrtenol | 0.54 | NA | 0.83*** | 0.71** | NA |
| Boring males (<i>n</i> = 14) | | | | | |
| Frontalin | NA | | | | |
| <i>endo</i> -Brevicomin | NA | NA | | | |
| <i>trans</i> -Verbenol | -0.31 | NA | NA | | |
| Verbenone | -0.28 | NA | 0.86*** | NA | |
| Myrtenol | -0.35 | NA | 0.95*** | 0.90*** | NA |

Sequential Bonferroni adjusted *P* values**P* < 0.05, ***P* < 0.01,****P* < 0.001

et al. 1976) were detected in much higher amounts than those derived by *de novo* synthesis (frontalin and *endo*-brevicomin; Vanderwel et al. 1992, Barkawi et al. 2003) in beetles that were dispersing from different trees in the vicinity and landing on the two trees that we sampled, as well as in boring beetles that we excised from one tree. The coefficients of variation among individuals were high in both cases, suggesting that the absolute metabolic cost associated with pheromone production might be trivial. However, it may be easier to produce large amounts of pheromones that are derived from host precursors compared to those synthesised *de novo*.

In other insects, pheromone production has been reported to have fitness costs. Male Hawaiian fruit flies, *Drosophila grimshawi*, produced sex pheromones for a greater proportion of their lives if they did not encounter females, and had shorter life spans as a result (Johanson et al. 2005), indicating adaptive phenotypic plasticity

depending on the environment they were in. Byers (2005) suggested that production of the alarm pheromone (E)- β -farnesene in the cotton aphid, *Aphis gossypii*, is associated with significant energetic cost because the amount produced increased with increasing body weight in adults who were genetically identical siblings, while small nymphs produced more pheromone relative to their body weight. Pheromone production by *I. pini* that arrived early in the attack sequence attracted predators and as a result, increased predation on late arriving beetles (Aukema and Raffa 2004).

High correlations between amounts of different pheromones produced by the same beetles (Table 3), particularly between pheromones that serve different functions and are derived from different metabolic pathways were detected in *D. frontalis*. Positive correlations in different pheromones in the same individuals were seen in a similar study in *D. ponderosae* and *I. pini* (Pureswaran 1999). This implies that the amount of pheromone that bark beetles can produce may be constrained by their metabolic or genetic potential. Both male and female *D. frontalis* had higher amounts of pheromones on landing than they did when they were boring in galleries (Table 1, 2), and both sexes differed in the pheromones they produced (Table 1). Once they were paired in galleries, females that had high amounts of the antiaggregation pheromone, verbenone, were paired with males that also had high amounts of verbenone and myrtenol (Table 3). This assortment indicates that pheromone production by one sex may influence the other, or that the local resin content around the gallery may determine the amount of pheromone beetles can produce.

Table 4 Correlations in pheromone amounts between paired female and male (*n* = 14) *D. frontalis* boring in the same gallery

| Females | Males | | | |
|------------------------|-----------|------------------------|-----------|----------|
| | Frontalin | <i>trans</i> -Verbenol | Verbenone | Myrtenol |
| Frontalin | 0.24 | 0.14 | 0.29 | 0.30 |
| <i>trans</i> -Verbenol | -0.06 | 0.53 | 0.54 | 0.56 |
| Verbenone | -0.23 | 0.64 | 0.76* | 0.72* |
| Myrtenol | 0.11 | 0.38 | 0.54 | 0.47 |

Sequential Bonferroni adjusted *P* values**P* < 0.05

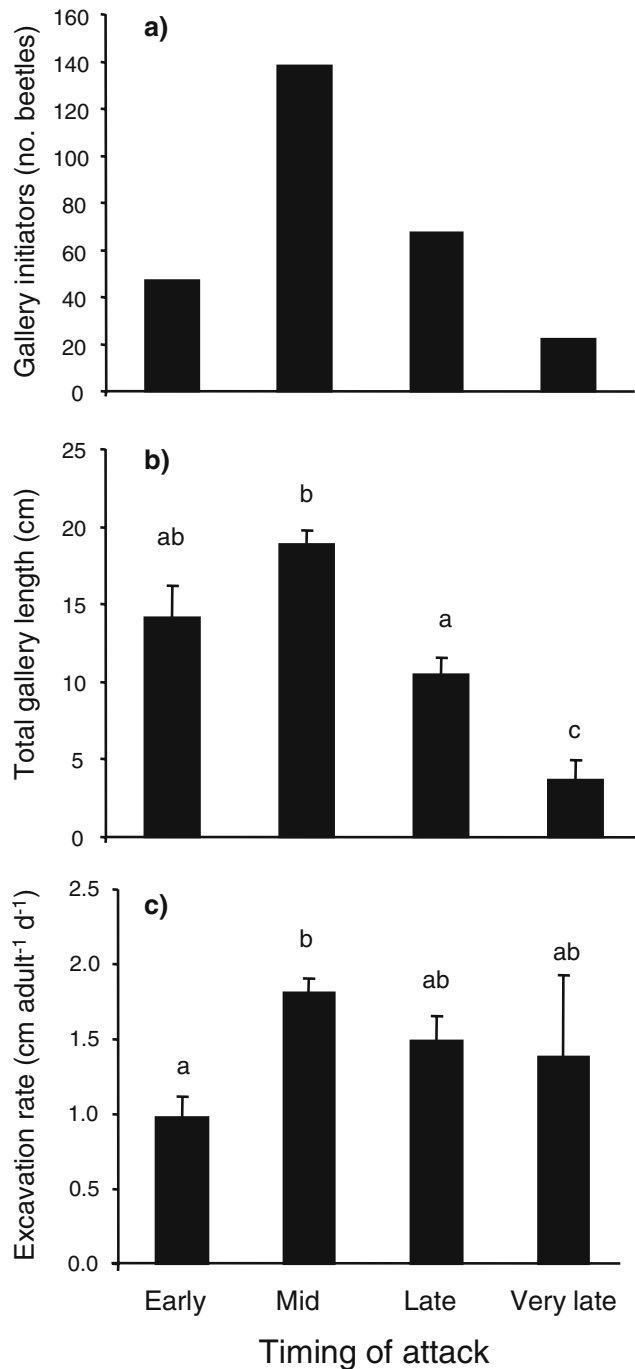


Fig. 2 **a** Number of *D. frontalis* that initiated galleries during two weeks on a 1.25 m long section of *P. elliotii*. **b** Total gallery length and **c** gallery length excavated per day by adults that joined the attack at different times. Values are means + SE. Bars with the same letter are not significantly different, REGW multiple comparisons test, $P < 0.05$

Timing of attack and beetle fitness

Most beetles attacked the focal study tree between the fifth and ninth of August, at the middle of the attack sequence (Fig. 2a). The first three beetles that initiated galleries before the 31st of July all drowned in resin,

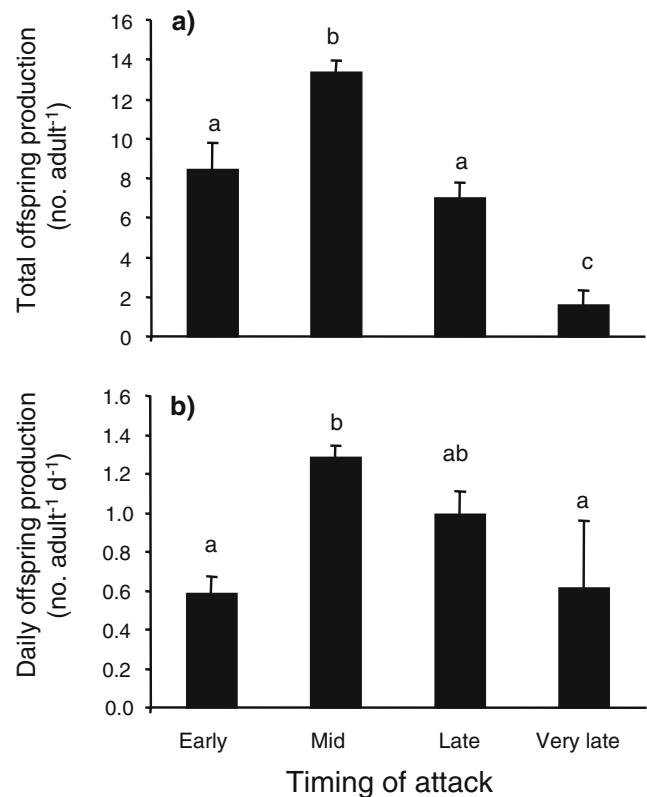


Fig. 3 **a** Total per capita offspring production and **b** daily per capita offspring production by adult *D. frontalis* that joined the attack on *P. elliotii* at different times. Values are means + SE. Bars with the same letter are not significantly different, REGW multiple comparisons test, $P < 0.05$

revealing the dangers of being pioneers. Although the total length of galleries excavated by beetles in the middle of the attack sequence was not significantly more than those of early arrivers, the length excavated per day that they were in the phloem was significantly greater. This suggests that beetles that arrived early in the attack sequence were slowed down in the first few days by tree defences, and concurs with earlier findings (Raffa and Berryman 1983), in which mountain pine beetles, *D. ponderosae*, did not make much progress in gallery excavation before aggregation occurred. Gallery length excavated per day by late and very late arrivers did not differ significantly from beetles that arrived either early or in the middle of the sequence, despite their total length being less than those of beetles that came in at the middle. This indicates that gallery excavation became easier and consumed less time as attack on the tree progressed.

As predicted, there was no difference in the fitness of early arrivers compared to late arrivers with respect to total offspring produced per capita, or with offspring produced per capita per day (Fig. 3a, b) implying that net reproductive costs and benefits tended to equalise fitness differences between the two groups. There was a high positive correlation between gallery length excavated per day, and number of offspring produced per day, confirming the findings of Clarke et al. (1979) that

the ability to excavate galleries quickly is an important component of fitness in *D. frontalis*. Beetles that arrived in the middle of the sequence achieved the highest fitness of the four categories. If there is in fact a “strategy” to timing arrival on a tree, the best payoffs are for beetles that arrive in the middle of the attack sequence.

Our data are consistent with the view that stabilising selection operates to favour beetles that initiate attack in the middle of the attack sequence, a few days after the first beetles arrive on the tree. These beetles avoid some of the initial costs of battling tree defences experienced by early arrivers, and they face less pressure from competition than late arrivers. This would be manifested by selection for most beetles to attack within a relatively small time frame culminating in synchronised mass attack, followed by synchronised emergence of brood in the next generation. Attack synchrony is a powerful phenomenon that enables beetles to successfully colonise trees by overcoming their defences easily, and contributes to outbreaks that are characteristic of bark beetle populations.

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